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FIRST TRIANNUAL REPORT (YEAR 3)

for period February 1, 1994 to May 31, 1994

Report Date: June 17, 1994

ONR Grant No. N00014-92-J-1244

**EVALUATION OF DRIED STORAGE OF PLATELETS FOR TRANSFUSION:
PHYSIOLOGIC INTEGRITY AND HEMOSTATIC FUNCTIONALITY.**

Principal Investigator:

**Arthur P. Bode, Ph.D.
East Carolina University
School of Medicine**

94-19360



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Attachments:

**Report from subcontract principal investigator, Marjorie S. Read,
Ph.D., The University of North Carolina at Chapel Hill.**

Abstract submitted to American Association of Blood Banks

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Administrative Activity:

Patent protection for commercialization of the manufacture and use of lyophilized platelets in transfusion medicine is still pending in U.S. Patent Court and internationally under the CPT. Armour Pharmaceutical Corp. has recently exercised its option to obtain a license for the intellectual property. The investigators of this subject grant will act as consultants to Armour to advise on scaling up of the research processing. This alliance of efforts will advance Specific Aim #4 farther than we had originally proposed.

The collaboration is underway with Dr. Morris Blajchman to test the human lyophilized platelets for correction of bleeding times in thrombocytopenic rabbits. Three different preparations, representing the platelets from a total of six units of blood, were processed and sent in April for Dr. Blajchman's analysis. A small delay has been encountered in that he must raise the titer of the rabbit platelet antiserum used in this model before commencing testing sometime this month.

Two manuscripts have been written to-date on current results, one stressing the in vivo findings in animal thrombosis models and the other stressing Baumgartner perfusion experiments of adhesion and activation. Both manuscripts are still being reviewed and edited in house in preparations for submission to the Proceedings of the National Academy of Science and the Blood journal. An abstract was submitted in this reporting period to the American Association of Blood Banks detailing the most recent data on the hemostatic effect of lyophilized platelets (see attached).

Scientific Progress:

The work at ECU has focused on long term storage of dried platelets at R.T. or 4°C desiccated, or at -70°C. The first such trial, conducted in Years 1-2 of this project, showed deterioration of surface glycoproteins and functionality after 9 months at R.T., but not at 4°C or -70°C even at 12 months. Another two preparations (one Para, one Perm) were put into storage on November 19, 1993, and evaluated this quarter after six months. The preparation treated with permanganate at 0.02% showed significant deterioration and disintegration of platelets upon reconstitution regardless of storage temperature. However, preparations with other strengths of permanganate should be tested before abandoning permanganate as an alternative stabilizer. The Para preparation (1.8% with 500 mM Trehalose) showed no such signs of deterioration at 4°C or -70°C, but the aliquots at R.T. already have reduced aggregation response to ristocetin, hypotonic shock response, AN-51 staining of GPIb, and the activation markers were up to 80% positivity. The evidence is compelling from this and the prior storage studies that storage at 4°C or -70°C is preferable to R.T. for long term stability.

An item of equipment purchased under the new grant (N00014-93-1-1034), the Xylum Clot Signature Analyzer (CSA) is being used as an adjunct to the Baumgartner perfusion chamber analysis of platelet adhesion under Specific Aim #3. The CSA provides an in vitro bleeding time test of platelets, including measures of platelet plug formation, procoagulant activity promoted by the platelets, and collagen-platelet interaction. We have tested five different Para-platelet preparations in this device under a variety of experimental conditions since its installation in April of this year.

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The results indicate a near normal response of the reconstituted platelets in plug formation and clot promotion, but a somewhat reduced collagen interaction (indicated by the time required to form an occlusive thrombus under high shear in a channel containing collagen fibrils) compared to normal fresh platelets. Further experimentation has suggested that the defect in collagen interaction is no worse than that seen in platelets from liquid blood bank concentrates stored 6-7 days.

The total number of human lyophilized platelet preparations made in this reporting period was fifteen. We are continuing to compare the effects of different preparation protocols on adhesion and activation in the Baumgartner chamber as well as other testing. The different types of preps analyzed were: Para-platelets stabilized for 45, 60, or 120 minutes, and permanganate-platelets stabilized at 0.01% or 0.02%. The most favorable results are still being obtained with 1.8% Para for 45-60 minutes as the stabilizer.

Attached to this report is the subcontract summary from Dr. Read at UNC-Chapel Hill describing data obtained on Specific Aims #1 and 2 in vivo. Future studies will continue along these same lines.

**Report period: February 1994-May 1994
University of North Carolina at Chapel Hill**

**Contract: UNC/ECU
Grant No. N0014-92-J-1244
The Office of Naval Research
Department of the Navy**

**Performance Site: University of North Carolina at Chapel Hill
Principal Investigator: Marjorie S. Read, Ph.D
Co-PI: Robert Reddick, MD**

Grant No. N0014-92-J-1244

Progress report for February 1994 to May 1994

The hemostatic and thrombogenic effects of the rehydrated platelets and the characterization of the surface antigens of rehydrated platelets (Specific aim #1 and The effects of rehydrated platelet transfusions of intravascular clotting (Specific aim #2)

We have been developing methods for labeling rehydrated and fresh platelets with fluorescein as well as with radioactive tags for use in hemostatic and thrombogenic studies. As noted in the previous progress report, we have labeled rehydrated platelets with fluorescence using Zynaxis PKH26. We have developed a way to label fresh platelets with the Zynaxis PKH26 dye by manipulating labeling conditions. This will allow us to monitor both fresh and rehydrated platelet infusates, providing similar control and experimental conditions. To insure that the labeling process did not change the hemostatic potential of the platelets, fresh PKH26 labeled and unlabeled platelets as well as rehydrated PKH26 labeled and unlabeled platelets were used for clotting studies in the Diagnostica Stago ST-4 machine. The clotting time for fresh canine unlabeled platelets was 80.4 ± 1.4 seconds. The clotting time for fresh PKH26 labeled platelets was 124.15 ± 4.0 seconds. The clotting time for rehydrated unlabeled platelets was 87.95 ± 2.0 seconds. The clotting time for rehydrated PKH26 labeled platelets was 90.15 ± 4.5 seconds. These studies suggest that the PKH26 label does not have a significant effect on the ability of rehydrated platelets to form clots. However, there is a notable difference in the clotting time of labeled fresh platelets and unlabeled fresh platelets.


In our continuing studies on the characterization of the surface antigens of rehydrated platelets, we have begun studies using ^{125}I labeled fibrinogen binding to rehydrated platelets in comparison to fresh platelets. Preliminary studies using fresh and rehydrated canine platelets suggest that rehydrated platelets can bind fibrinogen, but they only bind 66% of the amount of fibrinogen normally found binding to fresh canine platelets. We will continue these studies to confirm our preliminary findings.

In an attempt to circumvent the difficulties in labeling rehydrated platelets with isotopes (see previous progress report), we labeled fresh platelets prior to the fixation process. Platelets labeled with ^{51}Cr prior to fixation lost 67% of the label after 7 hours. Platelets labeled with ^{111}In prior to fixation lost 71% of the label after 7 hours. Fresh platelets labeled with ^{125}I prior to fixation lost 56% (Iodogen label technique) or 70%

(Iodobead label technique) after 18 hours of incubation in Normal Dog Plasma. This technique does not appear to be a suitable method for labeling our platelet preparations.

We have also been investigating another Zynaxis product, ^{125}I -PKH95, for radiolabeling rehydrated platelets. Thus far, *in vitro* studies suggest that the label binds rehydrated platelets effectively, with a slight leach of label typical in the radiolabeling of platelets with other compounds. In a typical experiment using canine rehydrated platelets, we found that after incubation in normal dog plasma for 24 hours, over 90% of the label was still found in association with the platelets. *In vivo* studies are in process using ^{125}I -PKH95 labeled rehydrated platelets.

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HEMOSTATIC PROPERTIES OF LYOPHILIZED PLATELETS IN TESTS OF BLEEDING TIMES. Arthur P. Bode, Marjorie S. Read, Robert M. Lust, Depts. of Pathology and Surgery, East Carolina Univ., Greenville, NC, and Dept. Pathology, Univ. of N.C. at Chapel Hill.

Background: We have previously shown that lyophilized platelets (L-Plt) retain properties of adhesion and activatability in the Baumgartner perfusion chamber (Trans 33:72S, 1993). Now we have analyzed L-Plt in two systems directly testing hemostatic function. **Study Design:** One is a prototype device simulating the Ivy bleeding time in vitro (IVBT) and collagen-induced thrombus formation (CITF) in recalcified whole blood (Xylum Clot Signature Analyzer: CSA); the other is an in vivo bleeding time in dogs on full clinical heart-lung bypass before and after infusion of L-Plt. **Results:** On the CSA, L-Plt gave an average ($n=4$) IVBT of 1 min 58 sec and a CITF of 73% versus 2 min 14 sec and 88% respectively for fresh platelets. Expired platelet concentrates gave indeterminate results because aggregates clogged the lines. IVBT > 6 min and CITF < 25% is typical of vWD patients. In two canine heart-lung bypass studies, the in vivo bleeding time improved from >15 min to 5-7 min after infusion of a bolus of $2-3 \times 10^{11}$ L-Plt. The corrected count increments were (#1) 88% and (#2) 47% based on estimated circulatory volume. **Conclusions:** These results demonstrate the hemostatic activity of L-Plt and their potential value in transfusion medicine.

Please retype title: HEMOSTATIC PROPERTIES OF LYOPHILIZED
PLATELETS IN TESTS OF BLEEDING TIMES.

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